The effects of ecological gradients on epiphytic benthic dinoflagellates found in Hawaiian waters.

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Abstract

Ciguatera fish poisoning is thought to be caused from the toxin producing dinoflagellate, *Gambierdiscus toxicus.* Many reports have shown an increase in ciguatera outbreaks in areas where there was previous disturbance to the reef. These disturbances open up new substrate for macroalgae and microalgae such as *G. toxicus* to settle and grow upon. Coastal development also has an impact on coral reef ecosystems. Development often increases freshwater input and nutrient levels into these ecosystems. To see how ecological gradients affect dinoflagellates, a field study was performed to test the hypotheses that nutrient enrichment and depth have an effect on the abundance of dinoflagellates. Although no differences were found in the nutrient enrichment study, *G. toxicus* was found in the shallow depth isobath at the study site. The hypothesis that lower salinity levels have an effect on the abundance of *G. toxicus* was also tested. Two strains of *G. toxicus* from two locations were cultured. The strain from Puako grew at a faster rate with greater maximum abundances over a broader range of salinities than the Kapoho strain. The strain from Puako grew in a salinity level as low as 17‰ which may suggest that the low salinity tolerance of *G. toxicus* and its ability to grow in shallow water could increase ciguatera outbreaks in areas with higher nutrient input and freshwater intrusion.

Introduction

Ciguatera fish poisoning is a serious problem for people who ingest reef fish primarily in tropical and subtropical regions. In Hawai'i, ciguatera has been reported on the islands of Kaua'i, O'ahu, Maui, and Hawai'i (Hawaii Department of Health 2002). Many species of fish caught on these islands harbor ciguatoxins, the neurotoxins known to cause ciguatera. These toxins are thought to be produced by the free-swimming, single celled dinoflagellates such as *Gambierdiscus toxicus* Adachi et Fukuyo, and other benthic species that are found living in coral reef ecosystems (Campbell et al. 1987). The ciguatoxin accumulates in the flesh and internal organs of fish by the consumption of the dinoflagellates found on many species of macroalgae. Randall (1958) hypothesized that the toxin enters the food web when the dinoflagellates are grazed upon by herbivorous fishes. The toxins produced by the dinoflagellates are concentrated in the food chain when herbivorous fish indirectly consume these dinoflagellates while grazing upon macroalgae. The herbivorous fish are then consumed by larger predatory fish, which increases the concentration of the toxin even further through biomagnification processes and biotransformation of the toxin. The toxin is passed up the food chain another level when humans catch these toxic fish (Holmes and Lewis 1994; Gollop and Pon 1992).

The outbreaks of ciguatera are random and unpredictable. More than 400 species of fish are known to have caused ciguatera fish poisoning in the past. What makes ciguatera even more difficult to understand is that the level of toxicity can vary from fish to fish and also from one location to another (Legrand et al. 1990; Tindall et al.1984). Fish that are known to be toxic in one area of an island may not be toxic in other areas of

the island. It is impossible to tell if a fish is toxic just by looking at it because the fish do not appear to have ill effects from the toxins (Lange 1994).

The Hawai'i State Department of Health investigated each reported ciguatera case during the 5 year period from 1984 to 1988. Out of the 462 cases throughout the state, the Kona coast on the island of Hawai'i was responsible for the most cases during this period. The reported cases from the Kona coast were also noted to have increased during this time period. No major seasonal variation in incidence of ciguatera was observed during the five years, although there was an increase between the months of July through September, and a decrease during the months of October through December (Gollop and Pon 1992). Many reports have shown an increase in ciguatera outbreaks in areas where there was previous disturbance to the reef. The destruction of coral colonies provides new surfaces for macroalgae to grow on, resulting in an increase in abundance of *G. toxicus* and other epiphytic, benthic dinoflagellates (Bagnis 1994). Gollop and Pon (1992) suggested that the large increase of incidences of ciguatera on the Kona coast may have been related to the construction projects and coastal development that had occurred early in the previous decade. Due to the expanding population, there are still major construction projects going on right now which could ultimately result in higher numbers of ciguatera cases than what was reported twenty years ago.

Shallow marine ecosystems near or directly seaward of these developed areas now face increasing nutrient enrichment from agricultural runoff or on-site sewage disposal systems and ground water nitrogen enrichment from suburban housing or hotel developments (Nixon et al. 2001). Coastal development increases the level of nutrients and freshwater intrusion. Coral reef ecosystems are very sensitive and require specific

conditions for reef growth. Coral reefs grow best in clear, warm, shallow, nutrient-poor waters, and in a salinity range of 30-40 parts per thousand. Sediment and nutrient run-off from developed areas can be harmful to reef growth and can alter the balance of the coral reef community. In healthy coral reef communities, the nutrient-poor water suppresses the rapid growth of algae and the grazers help keep them under control, allowing corals to successfully compete for space and light. If nutrient input is increased, the algae have the ability to take up these extra nutrients, grow very quickly, and ultimately out-compete the corals by growing over them (Castro and Huber 2000). Small changes in the ecosystem can have large impacts on community structure. This phase shift of coral abundance to algae abundance in coral reef ecosystems has been observed in reefs all over the world where reef degradation has taken place. The coral colonies that are damaged by natural disasters or anthropogenic destruction provide new substrate for the colonization of macroalgae including species that make up turf algae. Epiphytic, benthic dinoflagellates such as *G. toxicus* have been shown to have close association with macroalgae. These dinoflagellates have been found attached to dead coral substrates and on macroalgae in coral reef ecosystems (Yasumoto et al. 1980). Studies have found that various species of macroalgae show growth promoting ability toward benthic dinoflagellates (Carlson, 1984).

It is still unclear at what depth *G. toxicus* prefers to live on the reef. Many studies have collected the dinoflagellates in water as shallow as 0.5 meters, while others have collected them as deep as 30 meters (Grzebyk et al. 1994; Ballantine et al. 1988; Carlson and Tindall 1985; Gillespie et al. 1985; Tindall et al. 1984). This species may be found in a wide range of depths in other parts of the world, but its distribution is still not

well understood on Hawai'i's reefs. Salinity may also play an important role in the distribution of benthic dinoflagellates. Most ocean and coastal waters average 35 ppt, but in areas near river mouths or coastal development, the salinity is much lower due to the freshwater run off. The tolerance of lower salinities is not well understood in toxic dinoflagellates. The optimal range of salinity for many benthic dinoflagellates grown in culture is between 30 and 34 ppt (Morton and Norris 1990; Bomber et al. 1988). The decrease in salinity in nearshore areas is due to freshwater run-off from terrestrial systems. This freshwater run-off usually carries nutrients with it from these systems. It may be possible that the freshwater run-off is a barrier to growth of dinoflagellates, but it is not known if the increased nutrients may promote growth regardless of the decrease in salinity.

Coral reef destruction provides new substrate for algae and the increase of nutrient enrichment into these ecosystems has the potential for increasing the frequency of ciguatera outbreaks. To control or predict the appearance of ciguatera, further testing is needed to understand the processes in which ciguatoxin is introduced into the ecosystem. The main purpose of this study was to gain a better understanding of the ecological factors governing the presence of epiphytic benthic dinoflagellates such as *G. toxicus* so they are easier to locate in coral reef ecosystems around Hawai'i. The objectives of this study were to compare two different depth isobaths to find which isobath had a greater abundance of dinoflagellates. In addition, nutrients were introduced into the system to see how it affected the absolute and relative cell abundance of benthic dinoflagellates. For further understanding of *G. toxicus* ecology, a culture aspect was included in this study to find the salinity that promotes the greatest growth of cells.

Materials and Methods

FIELD STUDY

The site chosen for this experiment was Leleiwi Beach Park in Keaukaha on the East side of the island of Hawai'i (Figure 1). Within this site, the experiment focuses on two separate depths: deep (30-40 feet), and shallow (10-20 feet), and two fertilizer treatments (fertilized/non fertilized) for sampling. The sample substrates for this experiment consisted of calcium carbonate coral heads of the species *Porites lobata* that were collected from various beaches on the island. The surface area of the substrates ranged from about 250cm^2 to 850 cm^2. The treated sites consisted of coral heads that were altered to hold plant fertilizer. This was done by drilling a hole into the coral, large enough to insert a 50mL graduated polypropylene screw-capped centrifuge tube with drilled holes. Slow release fertilizer stakes were then placed into the tubes. The fertilizer used in this experiment was Jobe's fertilizer spikes for fruit and citrus trees with a ratio of 10-15-15 (Total N-P_2O_5–K_2O). The fertilizer stakes were enclosed in the tube by screwing on the cap and were not replaced during the experiment (Figure 2 A,B). The treated coral heads were then placed randomly within the two sampling depths. The untreated sites used for comparison also consisted of the coral head substrates, but these did not contain fertilizer stakes. Sampling was performed on each coral head by brushing the dinoflagellates off and collecting them onto a 60 micron mesh filter using a special sampling apparatus called "Dino-WeeVac" via SCUBA.

The "Dino-WeeVac", shown in figure 3, was used in this experiment to collect the dinoflagellates off of the coral substrates. At each sampling area chosen, the handle was turned ten times to brush the dinoflagellates off of the substrate. Once the brushing

was complete, the hand pump was squeezed and released ten times. This method was repeated at each of the sample replicates. A total of eight filters were used: two for the deep isobath that were treated, two for the deep isobath untreated, two for the shallow isobath treated and two for the shallow isobath that were untreated. Along with sampling using the Dino-WeeVac, water samples were collected at each sampling site for later analysis of nutrients, chlorophyll *a*, temperature, and salinity.

ONSHORE PROCESSING

To filter the water for the nutrient samples, a 47mm 0.2μm polycarbonate membrane filter was used. First 10mL of 10% Hydrochloric acid was filtered followed by 10mL of distilled water. Through the same filter, 15mL of the water sample was filtered and collected in a 15mL centrifuge tube for later analysis in the laboratory. This procedure was repeated so there were a total of two nutrient samples for each coral head substrate totaling 16 nutrient samples for each trip. A 25mm GF/F filter was used to filter 50mL of water for chlorophyll *a* analysis. The filter was removed from the filtering device and placed into a micro centrifuge tube for later analysis in the laboratory. At each sampling site, two 50mL water samples were filtered, giving a total of eight filters for each sampling trip. The temperature and salinity were measured by a hand held Model 85 YSI meter and recorded onto a data sheet. The filter from the Dino-WeeVac was removed from the cartridge and rinsed using water collected from the dive until the centrifuge tube was filled to 50mL. A one percent solution of gluteraldehyde was added to the samples for preservation. The samples were used to make slides for counting the relative and absolute abundance of the dinoflagellates.

LABORATORY ANALYSIS

The water samples collected for analysis of dinoflagellates were filtered through a 13mm/ 8 micron polycarbonate membrane filter to make microscope slides. Approximately 0.2mL was taken from the sample and placed into the Millerizer-a vacuum manifold system (Figure 4). The water was filtered through the Millerizer, and then with the pump off, two drops of diethanol (concentration of 4mg/10mL) were placed onto the filter. This was allowed to sit on the filter for approximately one minute and then filtered through. The tube and filter were rinsed with filtered seawater collected from the dive. The filter was then removed and placed onto a new, labeled slide. The slide was completed by placing two drops of immersion oil and a cover slip over the filter. The finished slide was then observed without the use of a microscope to see if the volume filtered was enough for a representative sample. This was determined by how thick the layer of debris was on the slide. If the slide was still clear, more volume was needed. The same procedure was followed for the slide preparation using double the volume of sample filtered. This was repeated until the slide has an adequate concentration of debris.

The nutrients and chlorophyll *a* involve the use of two different machines. The nutrients were analyzed using the Technicon Auto Analyzer II. It was used to measure the nitrate-nitrite, ammonium and phosphates in the seawater. The methods used to measure the nitrates and nitrites follow method No. 158-71W (1972) for the Technicon Auto Analyzer II. For ammonium, the methods used followed Gentry, C.E. (1988), and for phosphates, the methods used followed No. 155-71W for the Technicon Auto

Analyzer II. Chlorophyll *a* was analyzed using the Turner Designs Model 10-AU-005-CE Fluorometer following the EPA method 445.0 (Arar and Collins 1997).

Analysis of Slides

The slides prepared in the previous method were analyzed under an Olympus BX51 microscope at 200X magnification. The DAPI light filter on the microscope was used so the light had a bluish-purplish color making the diethanol stained cellulosic plates of armored dinoflagellates fluoresce. Counting and identifying was first done on slides that were previously counted for practice. The dinoflagellates were identified and counted with the help of Dr. Parsons and other resources such as Balech (1995), Chinain (1999), and Faust (1992, 1993a,b, 1996, 1999). The slides were examined by identifying the dinoflagellates and counting their absolute abundance and relative abundance. To find the absolute abundance of the different species of dinoflagellates, the following equation was used:

$$\#\text{ cells counted} \times \frac{1}{\text{vol. filtered}} \times \text{volume collected} \times \frac{1}{\text{area sampled}} = \frac{\#\text{ cells}}{\text{cm}^2}$$

The area sampled using the Wee-DinoVac is equal to 112 cm^2. To find the relative abundance, the number of cells for a certain species of dinoflagellates was divided by the total number of dinoflagellates cells counted on the entire slide. These results were then compared among the slides collected at each site.

Culture Study

A culture based study was also performed during this experiment. *Gambierdiscus toxicus* cells that were used in this experiment were sampled from Puako boat ramp on the west side of the Big Island and also from Kapoho on the east side of the Big Island.

The cells were collected prior to this experiment using the same methods used in the field study of this experiment. The separate strains were cultured in a modified Keller's media (Keller and Guillard 1985) and individually cultured. Once cell growth was established and several transfers had been made to new media, the dinoflagellates were chosen for use in this experiment. Several strains of dinoflagellates from Puako and Kapoho were grown prior to this experiment, but only one from each location was chosen. The dinoflagellates were chosen by making growth rate curves from the data that had been collected previously with the cultures and transfers (Figures 5 and 6).

Experiments were conducted with two clones of *Gambierdiscus toxicus*. Cultures were maintained in 50mL screw cap glass tubes filled with 15 ml of Keller's modified media. The cultures were maintained at 27°C and on a 12:12 hr light:dark cycle.

Medium for all cultures were made from filtered seawater of 35ppt. Salinity levels of 35, 29, 23,17, 14, 11, and 8 ppt were chosen for this experiment. Medium of lower salinity was made by diluting the seawater with deionized water. The equation used to calculate how much high salinity water to add was:

$$35\ x = 100\ x\ (\text{desired salinity}).$$

To figure out how much freshwater to add, the value from above was subtracted from the total volume of water needed for the medium. This water was then filtered through a sterile polycarbonate membrane filter for sterilization. The medium was made by adding 1 ml of concentrated Keller's modified media to 99 ml of filtered sea water.

Biomass was monitored daily between 1500 and 1700 hours by measuring *in vivo* fluorescence of each tube by inserting it directly into the Turner Designs Model 10-AU-005-CE fluorometer. For every reading, the dinoflagellates were removed from the

bottom of the tube and stirred up before the reading. The tube was then placed into the fluorometer and allowed to stabilize for one minute before the reading was taken.

Growth rates were found by calculating the slope of the log transformed data. The slope of the growth curve represented the change in cell fluorescence per day, therefore, indicating a increase or decrease in growth. To compare the Kapoho and Puako strains, the thirteenth day was chosen as the endpoint because both strains were still in exponential growth during this period. The maximum biomass was also compared among the strains. The highest fluorescence reading throughout the experiment was used for this value.

Statistical Analysis

The statistical program MINITAB was used to statistically compare the absolute abundance of dinoflagellates on the treated and non-treated sites with the separate depths using a two-way Analysis of Variance. This same procedure was used to compare the depth to the relative abundance values. Nutrients, chlorophyll, temperature, and salinity were each compared separately to depth, relative abundance of cells, and absolute abundance of cells using a two way ANOVA test. The growth rate and maximum biomass of the culture data were compared using a one way ANOVA using the Tukey's pairwise comparison when the data were normally distributed. Non-normal data were compared using a Nonparametric Kruskal Wallis test.

Results

Field Study-Dinoflagellate abundance

Dinoflagellates were found on all of the coral heads two weeks after deployment. The macroalgal growth after two weeks was very minimal, but conditions for

dinoflagellate growth were sufficient for settlement onto the dead coral substrates. In comparing the shallow versus deep depths, the average abundance of dinoflagellates found in shallow depths over a period of one month decreased from 5.44 cells · cm^{-2} to 0.94 cells · cm^{-2}, and the average abundance of dinoflagellates found in deep depths slightly increased from 1.19 cells · cm^{-2} to 1.47 cells · cm^{-2}. These values were not significantly different from one another (P=1.0). In the comparison of the fertilized coral heads versus the nonfertilized coral heads, the average abundance of dinoflagellates found on the treated coral heads decreased from 4.21 cells · cm^{-2} to 1.32 cells · cm^{-2} over a period of one month. This was also true for the nontreated coral heads which decreased from 2.42 cells · cm^{-2} to 1.10 cells · cm^{-2}, however, these values were not significantly different from one another (P=0.97). The relative abundance of each species was calculated and compared similarly to the average absolute abundance. The relative abundance of *Heterocapsa* sp. was significantly different for the depth variable (P=0.031) (Table 1 and Figure 7), having a higher abundance in the deeper depths. The abundance *Alexandrium affine* was also different between the two depth isobaths, (P=0.027) (Table 1 and Figure 8) but this species was only found in the deep depth isobath. The abundance of other species of dinoflagellates identified in this study were not significantly different in the depth, nutrient enrichment, and interaction variables (Table 1). Data from the field study was collected on only two occasions over a period of one month. The limited sampling was due to the conditions of the ocean. The conditions were calm when the coral heads were deployed, but after one month, the conditions were too rough at the study site for sampling and eventually the coral heads were lost or removed from their locations due to the high surf.

HYDROLOGICAL CHARACTERISTICS

Ammonia, nitrate, phosphate and silica were sampled prior to the initial deployment of the fertilized coral heads. The highest concentration for ammonia was 27.68 $\mu mol \cdot l^{-1}$, with a mean of 5.42 ± 7.39 $\mu mol \cdot l^{-1}$, nitrate was measured as 1.21 $\mu mol \cdot l^{-1}$, with a mean of 0.62 ± 0.30$\mu mol \cdot l^{-1}$, phosphate 0.63$\mu mol \cdot l^{-1}$, with a mean of 0.13 ± 0.16 $\mu mol \cdot l^{-1}$, and the highest value for silica was 127.02 $\mu mol \cdot l^{-1}$ with a mean of 35.15 ± 37.32 $\mu mol \cdot l^{-1}$. All of the nutrients were compared between the deep and shallow depths. Nitrate was found to be significantly different between deep and shallow ($P=0.032$, $F=5.66$) with a higher concentration in deeper depths, but no other nutrients were significantly different. No correlations were found between nutrients and salinity, nutrients and chlorophyll *a*, or chlorophyll *a* and salinity.

CULTURE STUDY

The strain of *G. toxicus* originally sampled from Kapoho did not grow in any of the four salinities. The cells became colorless and the cell fluorescence decreased over time. In the second trial, *G. toxicus* grew in all of the four levels of salinity. In comparing the slope of the logarithmic growth curve for each salinity over 13 days, the growth rate for *G. toxicus* growing in 17‰ was significantly different from those growing in 35, 29, and 23‰ ($P=0.004$). *G. toxicus* growing in 17‰ slightly increased, but the growth rate was much slower during this time period compared to the other salinity groups (Figure 9). The total growth period for *G. toxicus* from Kapoho was 25 days. During this period, the maximum biomass of *G. toxicus* grown in 17‰ was significantly lower than the maximum biomass of the other three salinity levels ($P=0.001$) (Figure 10).

The strain of *G. toxicus* originally from Puako grew in the four levels of salinity, but the rate of growth was not equal. The logarithmic growth curves over the 13 day period for the four different salinities were significantly different from one another (P=0.003). Growth rates for the dinoflagellates were different for those growing in salinity levels 35 and 23, and salinity levels 23 and 17 (Figure 11). The intervals for column level mean minus the row level mean of the Tukey's pairwise comparison table were very close to being significantly different between 35 and 17, 23 and 29, and 29 and 35‰, but all of these included zero. The total growth period for *G. toxicus* from Puako was 30 days. During this period, the maximum biomass of *G. toxicus* was significantly different between 35 and 17, 35 and 23, 29 and 17, and 29 and 23‰ (Figure 12). The values for the growth rate of the 17‰ group were much lower than in the three other salinity groups. The growth rates for 23, 29, and 35‰ were very similar to one another, but values for 23‰ were just below the two other groups.

G. toxicus from the 17‰ group were split up and dispersed into three lower salinity levels of 14, 11 and 8‰ along with another salinity level of 17‰. The dinoflagellates were removed from the original group during the stationary phase of growth. This second trial with the lower salinities was not successful. The 17‰ group declined after three days of growth, but the lower salinity groups did not grow at all.

The growth rate and maximum biomass of the two strains of *G. toxicus* were compared for each salinity group. The growth rates for the two strains growing in the 17 salinity level were significantly different from one another. *G. toxicus* from Puako had a faster growth rate than the strain from Kapoho. The other three salinity groups were not significantly different from one another; however, P was equal to 0.050 for the

comparison between the two strains growing in the 23 salinity level (Table 2). The Kapoho strain had a growth rate value less than the Puako strain (Figure 13). Figure 14 shows the growth curves for each of the salinity groups over the entire length of the experiment. The values for maximum biomass were significantly different for each of the four salinity groups (Table 2). The Puako strain had a higher abundance than the Kapoho strain for each salinity group (Figure 15).

Discussion

Although the field component of this experiment did not run as long as expected, valuable information was obtained during this short period of one month. The use of dead coral as a substrate for the growth of benthic dinoflagellates was successful. Several species of benthic dinoflagellates inhabiting Leleiwi were identified including *G. toxicus*, which was only found in the shallow isobath. Other studies that sampled *G. toxicus* from the wild also found them to be abundant at shallow depths ranging from 0.5m to 8m (Ballentine et al. 1988, Carlsons and Tindall 1985, Gillespie et al. 1985, and Tindall et al. 1984). The greater abundance of dinoflagellates at shallower depths may be due to the presence of macroalgae where they are commonly found as epiphytes. Although there were no significant differences in the abundance of dinoflagellates for the nutrient factor of the field experiment, the concentration of total nitrogen was greater in the deeper isobath prior to the nutrient enrichment so it may have been possible to see a difference in the abundance of dinoflagellates if the experiment was run for a much longer period.

The culture of *G. toxicus* provided important information about the effects of salinity on its growth rate. Significant differences were found between the two strains.

The strain from Puako seemed much hardier than the strain from Kapoho because it grew at a faster rate with greater maximum abundances over a broader range of salinities (Figures 8-14). The difference between the two strains may be due to differences in the conditions of Puako and Kapoho. These two locations are on different sides of the island; therefore, nutrient levels may be different. The Puako strain may be able to tolerate lower levels of salinity in order to take-up the same amount of nutrients that are available at Kapoho at higher salinity levels. The optimal salinity for both strains was between 29 and 35‰ which was similar to the salinity in which the original cells were collected from the wild. These salinity values were similar to what Bomber et al. (1988) found growing *G. toxicus* in culture. In their study *G. toxicus* grew best in 32‰, but also between 30 and 34‰, which is similar to the salinity range in this experiment. No other studies had been able to grow *G. toxicus* at a salinity level as low as 17‰. Although the lower salinity levels of 14, 11 and 8‰ did not grow, it may be possible to slowly acclimate the dinoflagellates to grow at these lower salinity levels.

The results of this experiment were significant to Hawai'i because it shows that *G. toxicus* can grow in areas with freshwater intrusion. Coastal development in Hawai'i increases the levels of nutrients and freshwater run off. The increase of nutrients promotes the growth of macroalgae which can cause death in coral reef ecosystems by growing over the corals (Castro and Huber 2000). Dinoflagellates such as *G. toxicus* have been shown to have close association with macroalgae (Carlson 1984; Yasumoto et al. 1980). The abundance of macroalgae affects the succession of the other reef organisms such as fish. If the abundance of macroalgae increases, the abundance of herbivorous fishes may also increase due to the abundance of food available. This phase

shift from coral abundance to macroalgae abundance has been observed in reefs all over the world where reef degradation has taken place and coastal development has occurred. The dead coral substrate opens up new habitat for not only for macroalgae, but also for toxic dinoflagellates that live amongst these algae. Coastal development is occurring at a faster rate than in the past due to the increasing population. The large scale coastal development occurring presently on the Kona coast of the Big Island of Hawai'i could potentially cause an increase in ciguatera cases due to the greater abundance of nutrients running off into reef ecosystems. Although there is an increase of freshwater coming into these systems, this study has shown that freshwater input is no longer a barrier for growth of *G. toxicus.*

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Table 1: The P values for Depth, Treatment of Nutrient enrichment or no treatment, and the Interaction of the two variables obtained from a two way ANOVA for the absolute abundance and the relative abundance of species of dinoflagellates identified in samples obtained from dead coral heads.
*P is significant when α=0.05

Species	Response Variable	Depth	Treatment	Interaction
Ostreopsis labens	Absolute abundance	0.274	0.739	0.494
	Relative abundance	0.704	0.496	0.266
Ostreopsis ovata	Absolute abundance	0.227	0.607	0.642
	Relative abundance	0.104	0.762	0.571
Scripspsiella sp.	Absolute abundance	0.397	0.518	0.767
	Relative abundance	0.874	0.458	0.447
Armored other	Absolute abundance	0.424	0.311	0.424
	Relative abundance	0.821	0.425	0.871
Coolia sp.	Absolute abundance	0.147	0.844	0.753
	Relative abundance	0.215	0.191	0.164
Gambierdiscus sp.	Absolute abundance	0.347	0.347	0.347
	Relative abundance	0.347	0.347	0.347
Heterocapsa sp.	Absolute abundance	0.098	0.707	0.437
	Relative abundance	0.031*	0.528	0.976
Alexandrium affine	Absolute abundance	0.100	0.146	0.146
	Relative abundance	0.027*	0.082	0.082
Prorocentrum concavum	Absolute abundance	0.347	0.347	0.347
	Relative abundance	0.347	0.347	0.347
Prorocentrum emarginatum	Absolute abundance	0.475	0.772	0.325
	Relative abundance	0.877	0.688	0.248
Prorocentrum mexicanum	Absolute abundance	0.415	0.415	0.294
	Relative abundance	0.401	0.401	0.303
Protoperidinium sp.	Absolute abundance	0.347	0.347	0.347
	Relative abundance	0.347	0.347	0.347

Table 2: The P values for the comparison of growth rate and maximum biomass between the Kapoho and Puako strains grown in salinity levels 35, 29, 23, and 17‰. *P is significant when α=0.05.

Salinity	Growth Rate	Maximum Biomass
17	0.001*	<0.001*
23	0.050	0.004*
29	0.405	0.028*
35	0.824	<0.001*

Figure Captions

Figure 1. Study site: Leleiwi beach park located in Keaukaha on the Island of Hawaii.

Figure 2. Treated coral head **A.** Treated coral head showing the fertilizer inside the 50mL tube. **B.** Completed treated coral head shown with the cap on.

Figure 3. The Wee DinoVac. **A.** Hand pump used to suck the water through the filter. **B.** Filter cartridge that holds a 50 micron mesh filter. **C.** Brass handle used to turn the brush. **D**. Container that fits over the substrate and keeps the sample from washing away. **E.** Brush used to remove the Dinoflagellates from the substrate.

Figure 4. The Millerizer. **A**. Pump used to suck the water through the filters. **B**. Filter tubes containing 13mm/8 micron polycarbonate membrane filters. **C**. Large flask used to catch the water filtered through the system.

Figure 5. Growth rate curves for successional series of *G. toxicus* collected from Puako Boat Ramp.

Figure 6. Growth rate curves for successional series of *G. toxicus* collected from Kapoho.

Figure 7. Interval plot for relative abundance of *Heterocapsa* sp. on the deep (d) and shallow (s) coral heads sampled at Leleiwi beach park.

Figure 8. Interval plot for relative abundance of *Alexandrium affine* on the deep (d) and shallow (s) coral heads sampled at Leleiwi beach park.

Figure 9. Logarithmic growth curves for *G. toxicus* strain from Kapoho grown in 35, 29, 23, and 17‰ medium.

Figure 10. Comparison of maximum cell fluorescence for *G. toxicus* strain from Kapoho grown in 35, 29, 23, and 17‰ medium.

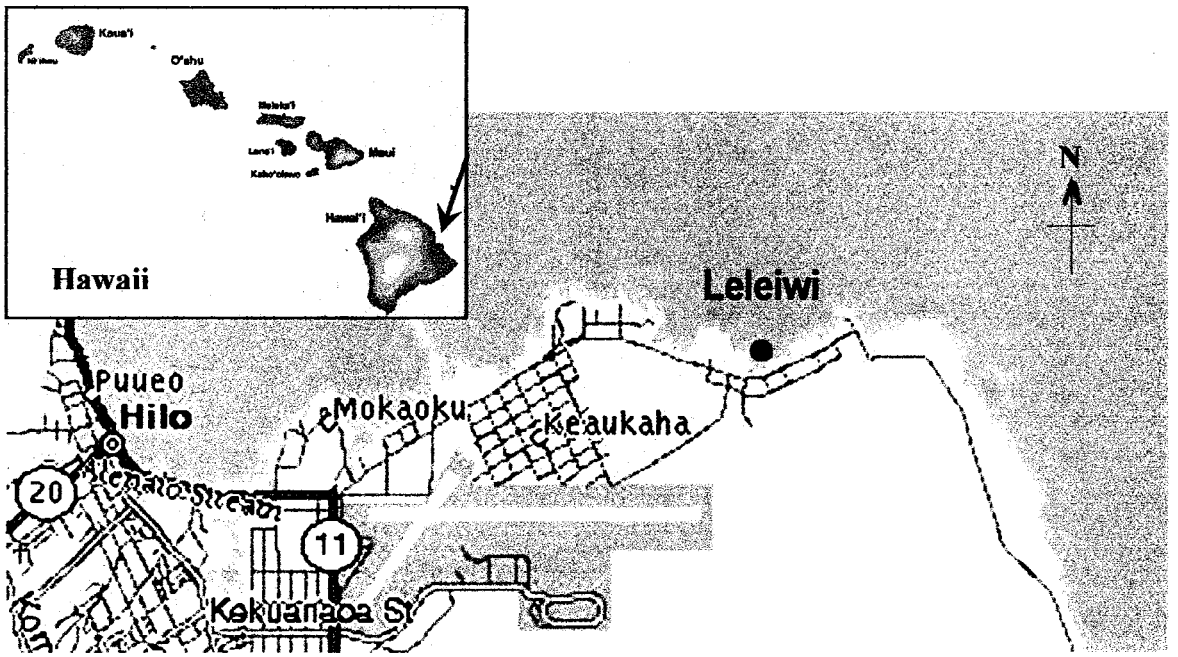
Figure 11. Logarithmic growth curves for *G. toxicus* strain from Puako grown in 35, 29, 23, and 17‰ medium.

Figure 12. Comparison of maximum cell fluorescence for *G. toxicus* strain from Puako grown in 35, 29, 23, and 17‰ medium.

Figure 13. Growth rate comparison for strains of *G. toxicus* Kapoho and Puako grown in 35, 29, 23, and 17‰ medium.

Figure 14. Logarithmic growth curves for *G. toxicus* strain from Kapoho and Puako grown in 35, 29, 23, and 17‰ medium over the total length of the experiment.

Figure 15. Comparison of maximum cell fluorescence for *G. toxicus* strain from Kapoho and Puako grown in 35, 29, 23, and 17‰ medium.



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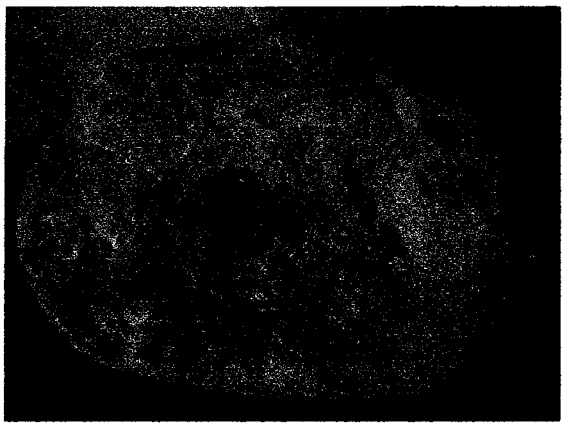

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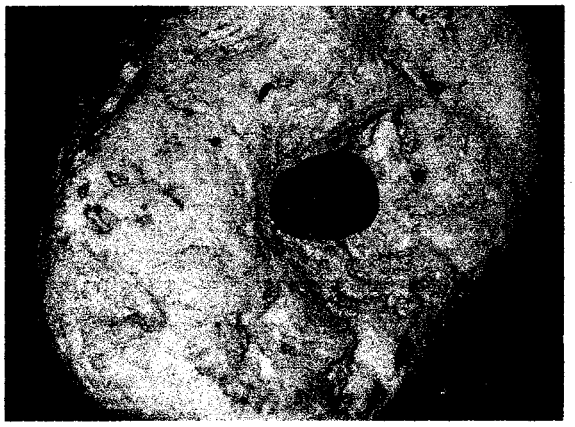

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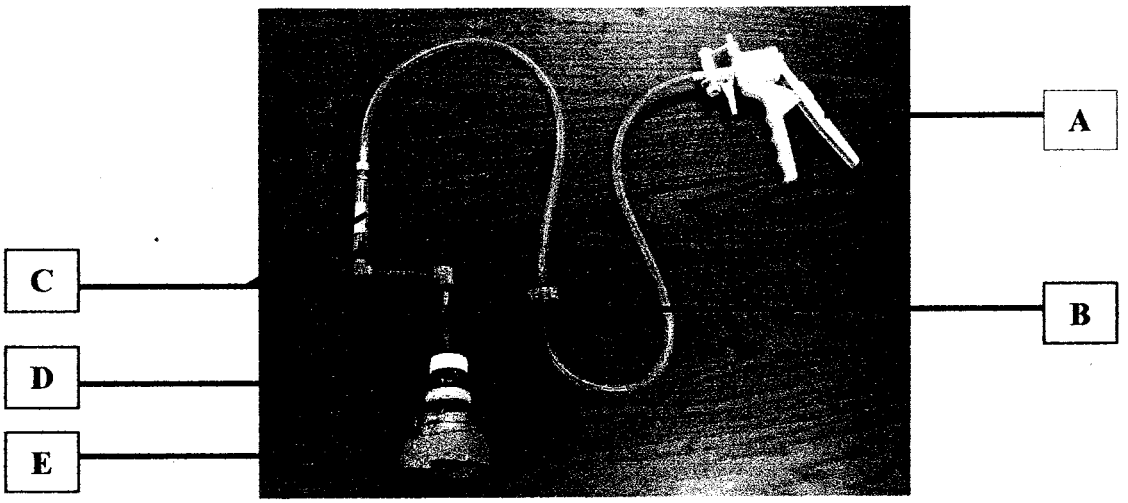

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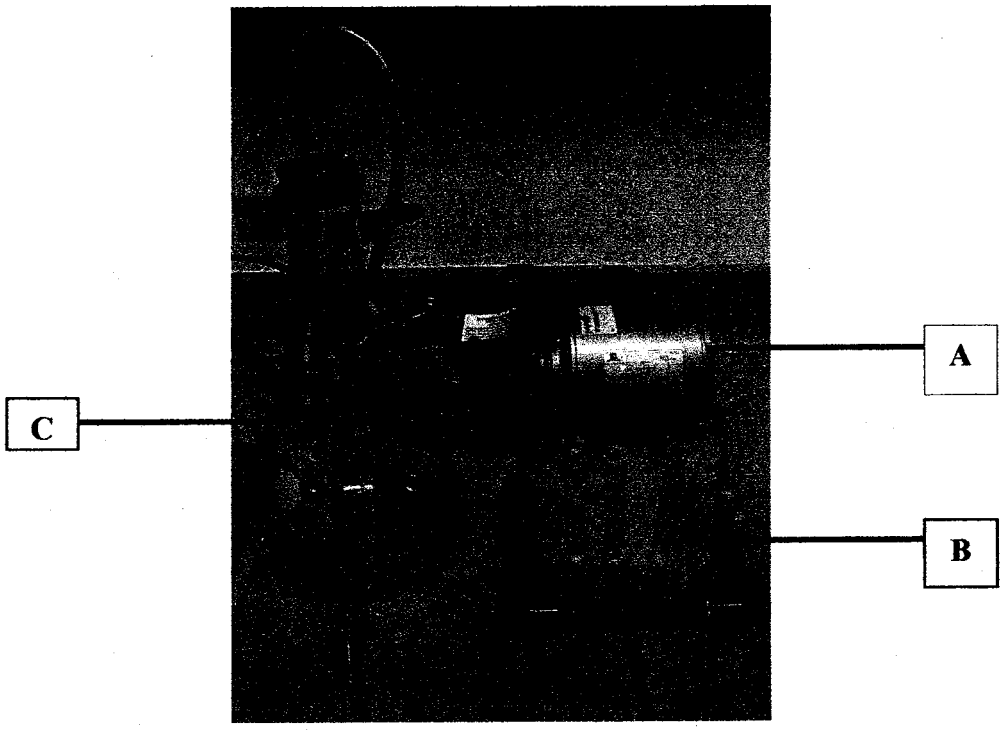
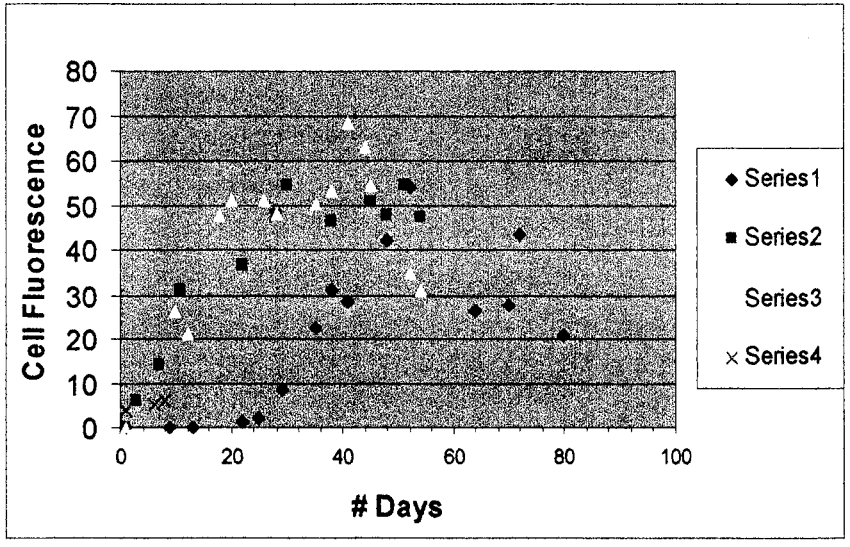


Figure 4



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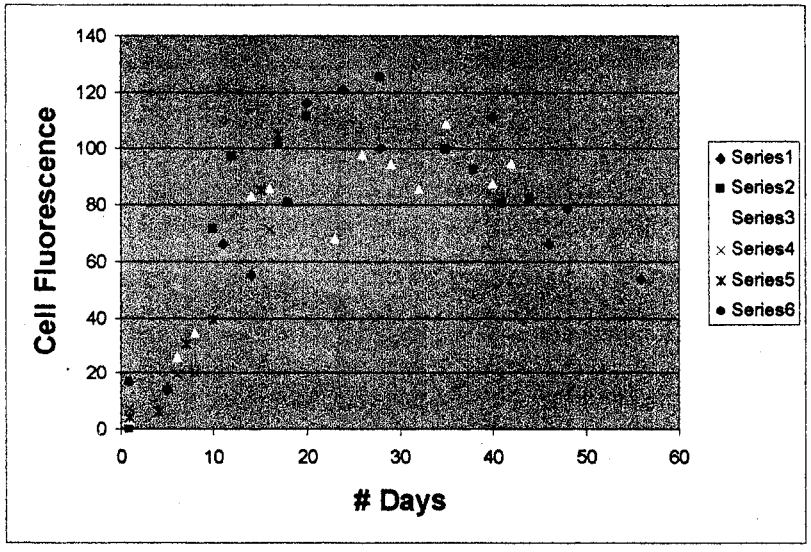
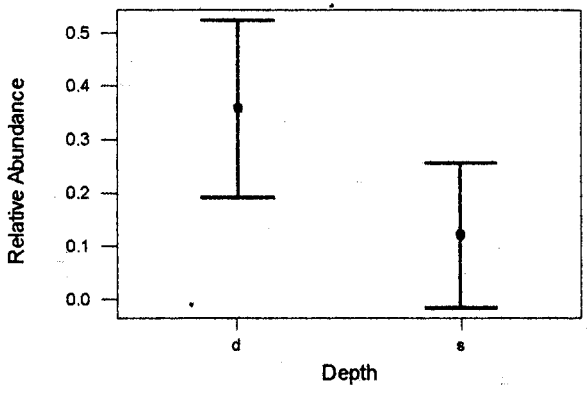


Figure 6



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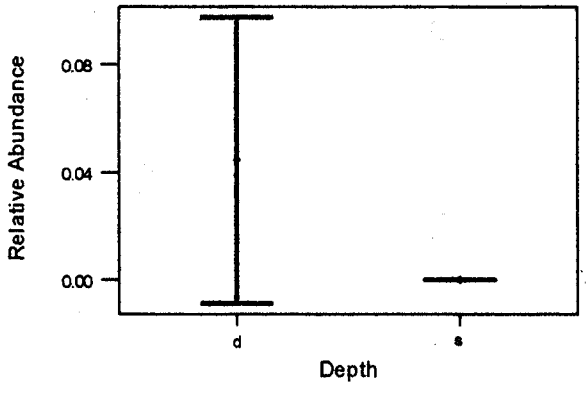

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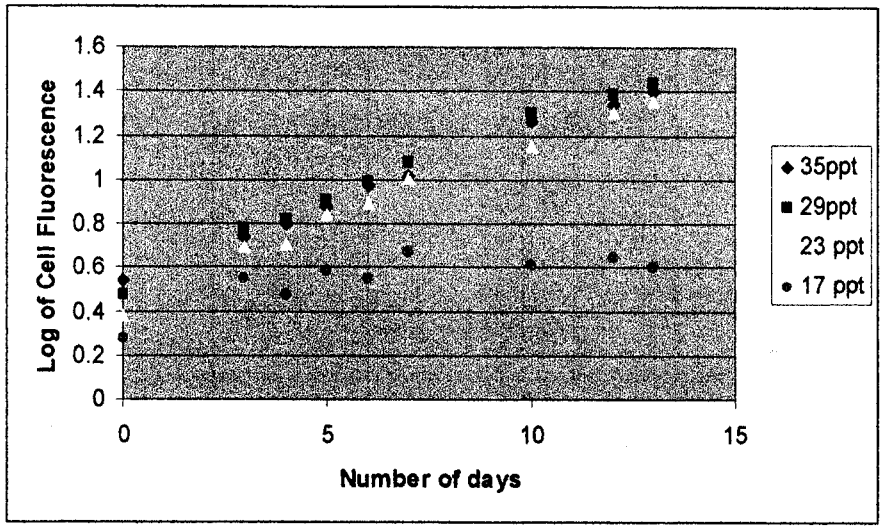

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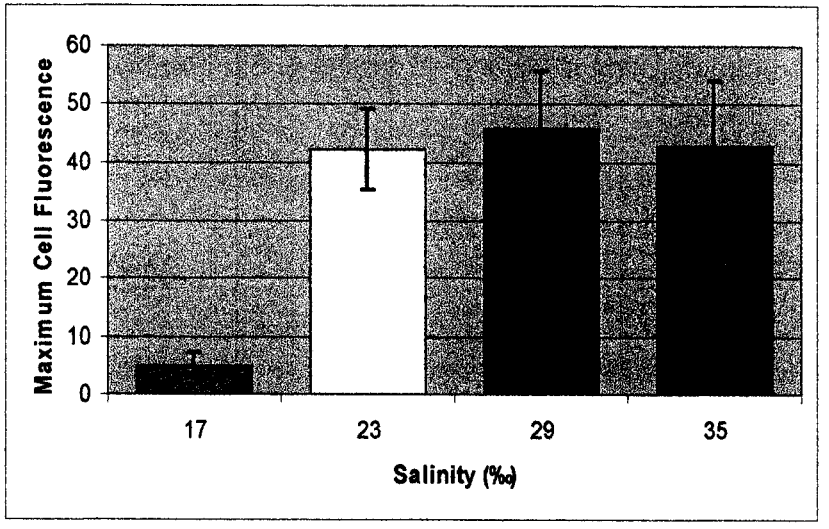

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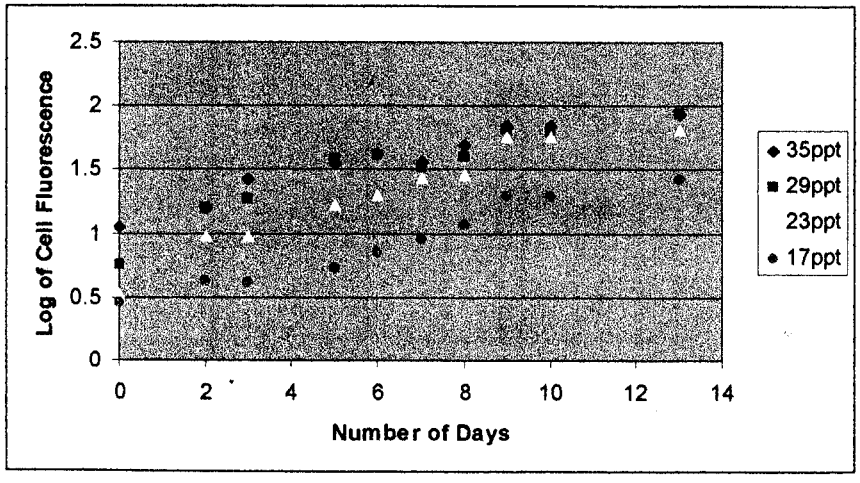

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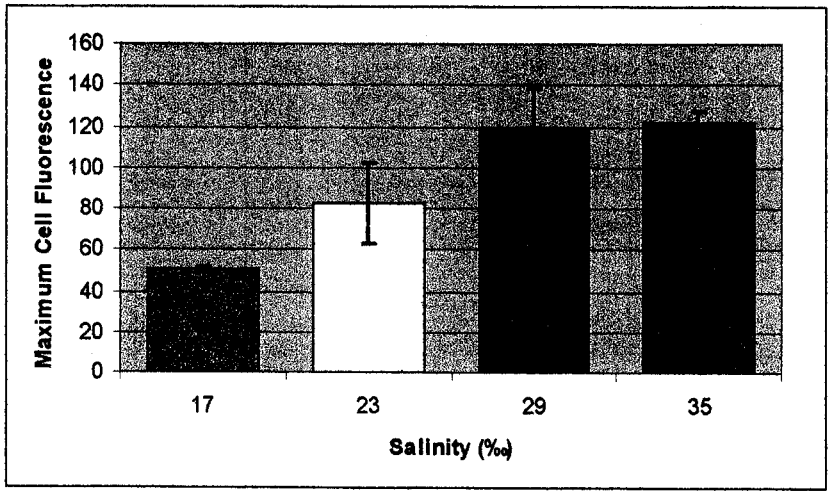

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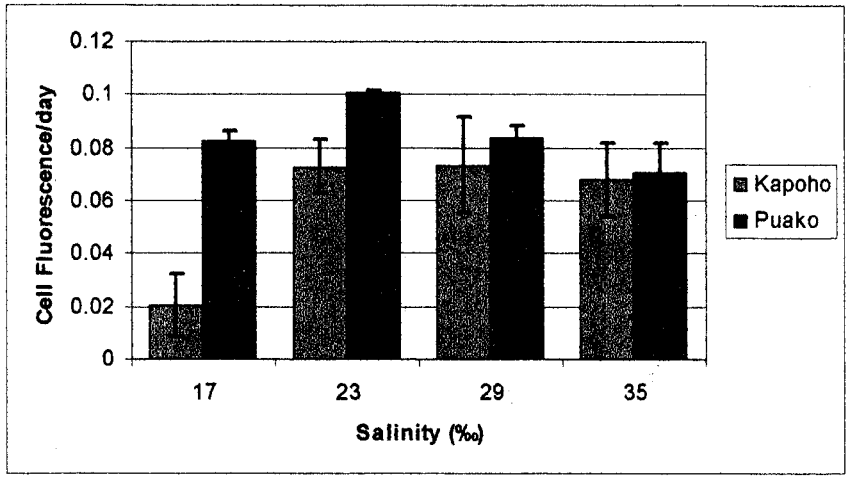

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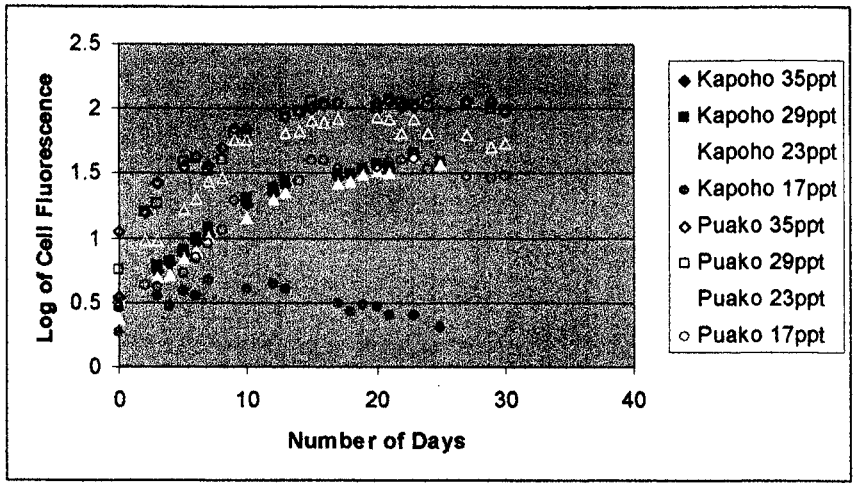

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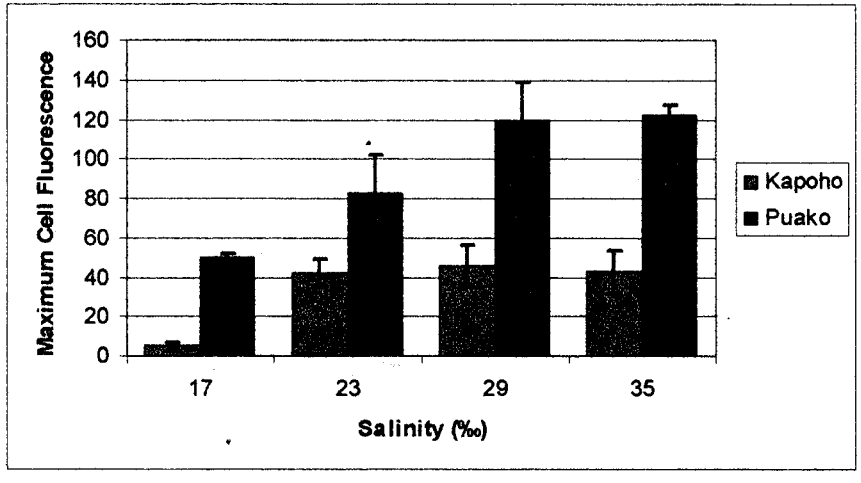

Figure 15.